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Abstract

The recombinant antibody AF122 binds to the acetylcholine receptor (AChR) α subunit expressed in murine muscle endplates and can be detected by immunofluorescence in paraformaldehyde (PFA)-fixed *tibialis anterior* cryosections.

Introduction

The AChRα (UniProt #P04756) is a subunit of the ligand-gated nicotinic AChR ion channel expressed at the neuromuscular junction. Binding of its ligand ACh leads to ion influx, to depolarization of the postsynaptic membrane and to subsequent contraction of the muscle. Here we report that the recombinant antibody AF122 detects by immunofluorescence the AChRα subunit in cryosections of the murine *tibialis anterior* muscle.

Materials & Methods

Antibodies: ABCD_AF122 (ABCD nomenclature, https://web.expasy.org/abcd/) targets the α subunit of the AChR. It was produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies) as mini-antibody with the antigen-binding scFv fused to a human IgG1 Fc. The synthesized scFv sequence (GeneArt, Invitrogen) corresponds to the sequence of the variable regions of the clone 637 (Graus *et al.,* 1997) joined by a peptide linker $(GGGGS)_4$. HEK293T cells (growing in DMEM GlutaMAX™ (Gibco, #31966) supplemented with 8% Fetal Bovine Serum (Gibco, #10270)) were transiently transfected with the vector coding for the scFv-Fc of the antibody. Supernatants (<5 µg/L) were collected after 4 days.

Antigen: The antibody AF122 was originally generated by a phage display selected for reactivity against the human AChR (UniProt #P02708). The phage display Fab library was generated from thymic lymphocytes derived from two Myasthenia gravis patients (Graus *et al.,* 1997). *Tibialis anterior* was isolated from C57BL/6 mice and rinsed in PBS. The muscle was embedded in OCT (Tissue-Tek, #4583) and cryosectioned at 10 µm using a Hyrax C60 cryostat (Zeiss).

Protocol: Cryosections were fixed with 4% (w/v) PFA supplemented with 0.1% Triton X-100 an goat serum. Subsequently, sections were ir the following primary antibodies overnight Fluor 647-conjugated α-bungarotoxin (1: Fisher Scientific, #B35450), mouse anti-r 200 (1:300, Sigma, #N0142) and the und monoclonal antibody AF122. Sections were in PBS and incubated with Alexa Fluor 488 anti-human and Alexa Fluor 555-labeled go secondary antibody overnight at 4°C Technologies, #A11013, #A28180). Counte performed using SlowFade Gold antifade DAPI (Invitrogen, #S36936). photomicrographs were captured with a confocal laser scanning microscope (Heerbrug, Switzerland) equipped with argo lasers using the 40x objective (oil NA1.25). Images were processed and merg imaging software (Bitplane, Zurich, Switzer

Results

The AF122 antibody staining in the n endplates of the murine *tibialis anterior* col bungarotoxin (Fig. 1), which binds with hi the AChRα subunit (Tzartos *et* Neuromuscular endplates are identified by of AChRα in the proximity of nerve fibers i Thus, AF122 recognizes the AChRα in fixed muscle endplates.

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(Electron Microscopy Sciences, #15710) in 0.1 M phosphate buffer, pH 7.4, for 10 min at room temperature, washed in PBS, and blocked with PBS

Conflict of interest

The authors declare no conflict of interest.

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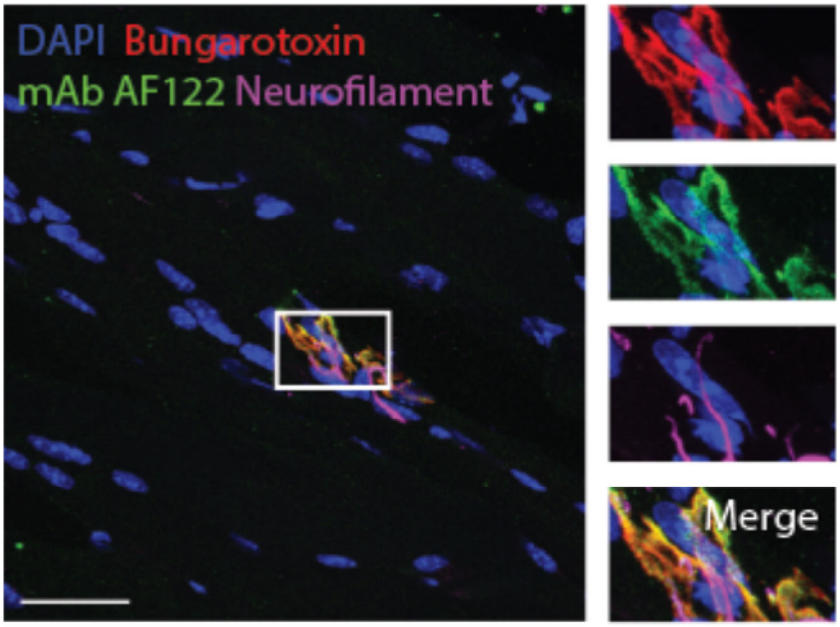

Fig. 1. The AF122 antibody recognizes the AChR in the murine *tibialis anterior* muscle. Muscle endplates are identified by neurofilar
AChRs are visualized by binding of bungarotoxin. AF122 in green, bungarotoxin in red, neurofilament in purple and DAPI in blue. Sca

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